EXPRESSION OF A DNA REPLICATION GENE CLUSTER IN BACTERIOPHAGE T4: GENETIC LINKAGE AND THE CONTROL OF GENE PRODUCT INTERACTIONS

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ABSTRACT

The results of this study bear on the relationship between genetic linkage and control of interactions between the protein products of different cistrons. In T4 bacteriophage, genes 45 and 44 encode essential components of the phage DNA replication multiprotein complex. T4 gene 45 maps directly upstream of gene 44 relative to the overall direction of reading of this region of the phage chromosome, but it is not known whether these two genes are cotranscribed. It has been shown that a nonsense lesion of T4 gene 45 exerts a cis-dominant inhibitory effect on growth of a missense mutant of gene 44 but not on growth of phage carrying the wild-type gene 44 allele. In previous work, we confirmed these observations on polarity of the gene 45 mutation but detected no polar effects by this lesion on synthesis of either mutant or wild-type gene 44 protein. In the present study, we demonstrate that mRNA for gene 44 protein is separable by gel electrophoresis from gene 45-protein-encoding mRNA. That is, the two proteins are not synthesized from one polycistronic message, and the cis-dominant inhibitory effect of the gene 45 mutation on gene 44 function is probably expressed at a posttranslational stage. We propose that close genetic linkage, whether or not it provides shared transcriptional and translational regulatory signals for certain clusters of functionally related cistrons, may determine the intracellular compartmentalization for synthesis of proteins encoded by these clusters. In prokaryotes, such linkage-dependent compartmentation may minimize the diffusion distances between gene products that are synthesized at low levels and are destined to interact.

DNA replication of bacteriophage T4 in infected Escherichia coli hosts requires the functioning of a large number of phage-induced proteins [see Mathews et al. (1983) for recent reviews]. Several of these proteins are encoded by a group of genes contained within a 25,000-base pair (25 kb) segment of the T4 DNA chromosome (about 166 kb) that includes the structural genes for T4 DNA polymerase (gene 43) and for three of its accessory proteins (genes 45, 44 and 62). T4 genes 44 and 62 encode the subunits of a single-stranded DNA-dependent ATPase that has been shown to stimulate the activity and processivity of the phage polymerase in vitro (Piperno and Alberts 1978). The T4 gene 45 protein further enhances the gene 44/62 ATPase effect on DNA polymerase (Piperno, Kallen and Alberts 1978). All four proteins are essential for in vivo replication. In addition, the gene 45 protein interacts with

FIGURE 1.—A physical map of the T4 gene 45-44-62 cluster showing the positions of neighboring gene regA, the 5'-terminal region of gene 43 and the 3'-terminal region of gene 46. The direction of genetic reading of all genes within this cluster is from right to left, i.e., counterclockwise relative to the circular T4 genetic map. Information about the positions of restriction enzyme sites (vertical arrows) was derived from analysis of cloned fragments of the genetic regions shown. The cloned fragments were also used in marker rescue experiments to determine the locations of the nonsense and ts mutants shown (see MATERIALS AND METHODS). Physical distances (map units in base pairs) are shown relative to the initiator codon for gene 45 mRNA (zero point on the scale), which was derived from the DNA sequence determinations of Spicer and Konigsberg (1983). The following symbols are used to represent restriction endonucleases: A, Aul; C, Clai; E1, EcoRI; H, HindIII; P, PslI; S, SphI; X, XhoI. The dashed area where an Aul site maps, represents a 76-nucleotide sequence that separates the termination codon (UAA) of gene regA from the gene 43 AUG initiator.

The positions of T4 genes 45, 44 and 62 in relation to neighboring genes on the phage chromosome are illustrated in Figure 1. All five genes designated in the figure are transcribed in the counterclockwise (gene 46 to gene 43) direction relative to the circular T4 genetic map (for additional references see Wood and Revel 1976; Karam, Bowles and Leach 1979); however, it is not yet clear to what extent these genes share transcriptional initiation and termination signals. Genetic studies seem to indicate that T4 gene 43 (DNA polymerase) is regulated independently of the other genes in the cluster, both at the transcriptional (Russell 1973; Miller et al. 1981) and translational (Karam and Bowles 1974) levels. The protein product of T4 gene regA is a translational inhibitor that regulates mRNA utilization and decay for several T4 genes including genes 45, 44, 62 and the regA gene itself (autogenous regulation) (Cardillo, Landry and Wiber 1979; Karam, McCulley and Leach 1977; Karam et al. 1981, 1982). Much of the region that encompasses genes 45, 44, 62, regA and 43 has now been sequenced at the DNA level (Spicer and Konigsberg 1983; M. Trojanowska and J. D. Karam, unpublished data) and, although some of the segments contained therein resemble known E. coli promoter sequences, no functional transcriptional initiation sites
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have been mapped within this genetic cluster either genetically or biochemically.

Two types of genetic polar effects have been described for nonsense mutants of the T4 gene 45-44-62 region. In one type of effect, two T4 gene 44 amber (am) mutations (44amN82 and 44amE4408, Figure 1) diminished gene 62 protein synthesis drastically, suggesting that genes 44 and 62 are cotranscribed and translationally coupled (KARAM, BOWLES and LEACH 1979). Another, more unusual form of polarity is exhibited by the T4 gene 45 nonsense lesion 45amE10 (Figure 1). This mutation does not affect synthesis of either gene 44 or gene 62 protein, but does exert a strong cis-dominant inhibitory effect on the growth of the gene 44 mutant 44tsB110 (KARAM, BOWLES and LEACH 1979). In the results to be described here, we show that the gene 44 and gene 45 proteins are translated from two separate T4-induced mRNA populations and that the mutation 45amE10 abolishes the gene 45 protein-encoding activity without affecting translational activity of the gene 44 protein-encoding mRNA fractions. Based on our previous genetic studies (KARAM, BOWLES and LEACH 1979; BOWLES and KARAM 1979), the results to be presented here and on information about nucleotide sequences near the gene 45-44 junction (SPICER and KONIGSBERG 1983), we propose that genes 45 and 44 are expressed independently of each other, but that their close linkage provides an in vivo compartmentalization that facilitates the interaction of their protein products. Such linkage-dependent localization of cellular sites for interactions between functionally related proteins may be a feature common to all prokaryotes.

MATERIALS AND METHODS

Bacterial and phage strains: The E. coli and T4 phage strains that were used in this study are listed in Table 1. E. coli CR63 was used for the preparation of phage lysates, E. coli B was used as host in experiments involving the extraction of phage-induced RNA and proteins, E. coli MRE600 was the source for the cell-free extracts (S30) used in the in vitro translation assays and E. coli K802 was used as the host for recombinant plasmids. All of the T4 strains that were used for analyses of expression of the gene 45-44-62 cluster carried nonsense lesions in phage genes 32 (32amA453), rIIB (rIIB ochre UV375), and 42 (42amN122) and the regA gene mutation, regAR9. The use of the gene 32, rIIB and 42 lesions allowed for better visualization in SDS gel electrophoresis of the gene 44 protein (which comigrates with the gene 32 and rIIB proteins) and gene 45 protein (which comigrates with the gene 42 protein). The regAR9 mutation causes increased stabilization of a subclass of T4-induced transcripts and hyperproduction of their translation products (KARAM and BOWLES 1974). The media and methods that were used for the preparation of phage stocks, labeling of phage-induced proteins with radioactive amino acids and analysis of labeled proteins by SDS-slab-gel electrophoresis have been described (KARAM, MCCULLEY and LEACH 1977).

Physical and genetic mapping of the T4 gene 45-44-62-regA-43 cluster (Figure 1): The relative positions of the genes in this cluster have been established [see WOOD and REVEL (1976) and MATHEWS et al. (1983) for reviews]. The relative positions of specific mutations have been determined by a combination of genetic and biochemical methods (KARAM, BOWLES and LEACH 1979). Restriction enzyme mapping was carried out on fragments of this T4 DNA segment that had been cloned in the plasmid pBR322. The fragments were derived from a λ-imm21-T4 recombinant phage named φNM761-4 that was given to us by NOREEN MURRAY. The genetic properties of φNM761-4 will be described elsewhere (J. D. KARAM and M. DAWSON, unpublished data). Standard agarose gel electrophoretic methods (MANIATIS, FRITSCHE and SAMBROOK 1982) were used in analyzing products of restriction enzyme digestion of the plasmid-borne T4 DNA fragments. E. coli
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<td>CR63</td>
<td>K-12, SupD60, λR (serine-inserting amber suppressor)</td>
<td>R. S. EDGAR</td>
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<td>K802</td>
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<td>MRE600</td>
<td>Wild-type strain low in ribonuclease activity</td>
<td>E. T. YOUNG</td>
<td>CAMMACK and WADE (1965)</td>
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<tr>
<td>B⁺</td>
<td>Wild-type (Sup⁺) B strain</td>
<td>L. GOLD</td>
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**Phage T4 mutant**

- 32amA453: Gene 32 UAG mutant
- 42amN122: Gene 42 UAG mutant
- 44tsB110: Gene 44 missense mutant
- 44amN82: Gene 44 UAG mutant
- 44amE4408: Gene 44 UAG mutant
- 45amE10: Gene 45 UAG mutant
- rII8UUV375: Gene rII8 UAA mutant
- regAR9: Gene regA mutant

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strains carrying specific recombinant plasmids were also used as hosts in marker rescue tests that assigned T4 mutations to specific DNA segments. In these tests, about 0.05 E. coli K802 cells (at 10⁹/ml) were mixed with 0.05 ml of mutant phage solution (at 5 × 10⁸/ml), and after a 15-min incubation at 30° the mixture was spotted onto a lawn of E. coli B⁶ cells. Lysis or plaque formation within the spot after overnight incubation (at 30° for am mutants and 42° for ts mutants) was indicative of recombination between the infecting phage and the T4 DNA insert of the plasmid. Cells carrying the pBR322 cloning vector were used in control tests.

**Growth conditions for the isolation of T4-induced RNA:** RNA was purified from E. coli B⁶ cultures that had been grown and infected with the appropriate phage mutants at 30° in M9 medium. Cell cultures grown to a density of about 3 × 10⁹/ml were concentrated to 2 × 10⁹/ml in M9 medium, equilibrated to 30° for 10 min in a reciprocating shaker bath and then infected at a multiplicity of 12 with the desired phage suspended in M9 at a volume equal to that of the cell culture. Successful infections were monitored via SDS-gel electrophoretic analyses of ¹⁴C- or ⁳²P-labeled proteins prepared from aliquots of the infected culture. At 8 min postinfection, the E. coli RNA polymerase inhibitor rifampicin (catalog no. R 3501, Sigma Chemical Company, St. Louis, Missouri) was added to the infected cultures at a final concentration of 200 µg/ml, and the cultures were aerated in the presence of the drug for 35 min before their RNA was extracted. In T4 regA⁰ infections, most T4-induced "early" mRNAs are degraded during the incubation with rifampicin, but in regAⁱ infections a subpopulation of these transcripts is not destroyed and remains active in protein synthesis (Karam, McCulley and Leach 1977).

**Isolation of T4-induced early RNA:** Under the conditions used for our studies, the infection of E. coli B⁶ (sup⁰) with the T4 32-H11-42-regA⁰ multiple mutants yielded mostly early phage RNA, as was judged by the lack of synthesis of any "late" phage proteins in vivo. Infected cultures (usually 1 liter at 1 × 10⁹/ml) were poured over ice to decrease metabolic activity, and cells were harvested by centrifugation at 11,000 × g for 5 min. Cell pellets were each washed twice in 50–100 ml of chilled TK buffer (0.01 M Tris, pH 7.5, 0.01 M KCl) and then suspended in a volume of the buffer corresponding to 2% of the original culture volume. The suspension was added DNase I (catalog no. 104 132, Boehringer-Mannheim, Indianapolis, Indiana) to a final concentration of 40 µg/ml, disodium EDTA to 0.01% and lysozyme to 0.8 mg/ml. The mixture was incubated at room temperature for 10 min, magnesium acetate (to 0.02 M) was added and incubation was continued for an additional 5 min. The cell suspension was twice rapidly frozen in a dry ice-acetone bath and thawed and then made 0.1% in SDS and 0.2 M in sodium acetate, pH 5.2. The cleared suspension was extracted twice with an equal volume of phenol that had been equilibrated with TK buffer. The final aqueous phase was made 0.2 M in sodium acetate, pH 5.2, and 2.5 volumes of 95% ethanol (at −20°) were added. RNA was allowed to precipitate at −20° overnight before it was pelleted by centrifugation, washed with cold 70% ethanol and dried under vacuum. The dried RNA pellets were stored at −20° until used.

**Analysis of purified T4-induced RNA by preparative gel electrophoresis:** T4-induced RNA was fractionated by a preparative gel electrophoresis procedure similar to that described by Hagen and Young (1974). We used an apparatus that was purchased from Savant Instruments, Inc., Hicksville, New York (model PAG 15WC). One to 1.2 mg of total RNA was dissolved in 90% dimethylsulfoxide in a volume of 0.5 ml and heated to 37° for 10 min to facilitate disaggregation of the nucleic acid. The sample was then layered on a 1-day-old preelectrophoresed cylindrical (1.5 × 5.4 cm) gel column consisting of polymerized 2.36% acrylamide/0.5% agarose in RNA electrophoresis buffer (0.04 M Tris-acetate, pH 7.8, 0.02 M sodium acetate, 0.002 M sodium EDTA, 0.1% w/v SDS). Preelectrophoresis of the composite gel was carried out at 10 mA for 1 hr at room temperature with buffer flowing through the elution chamber of the apparatus at the rate of 0.1 ml/min. Sample fractionation was carried out under the same conditions except that electrophoresis was for 24–30 hr. RNA elution was monitored by eluant absorbance at 254 nm, and 2-ml fractions were collected. Each fraction was mixed with 40 µg of E. coli tRNA, 0.2 ml of 2 M sodium acetate (pH 5.2) and 5 ml of 95% ethanol at −20°. The RNA pellets that formed after overnight incubation of the ethanol-precipitated fractions at −20° were collected by centrifugation at 5000 × g for 30 min, washed in 70% ethanol, dried under vacuum and each dissolved in 0.04 ml of sterile water prior to use in *in vitro* translation assays.

**In vitro translation assays:** *In vitro* translation of RNA was carried out in S30 extracts of E. coli MRE600 that were prepared by the method of Wilhelm and Haselkorn (1971). The translation...
assays used were similar to those described by HAGEN and YOUNG (1973), except that incubation of extracts prior to the addition of RNA (preincubation step) was carried out for 10 min at room temperature in the presence of 0.001 M CaCl$_2$ and 560 units/ml of micrococal nuclease (catalog no. 107 921, Boehringer-Mannheim); EGTA (0.002 M) was added to inactivate the nuclease at the end of the 10-min preincubation period. The RNA to be translated was dissolved in 0.04 ml of H$_2$O containing 5 μCi of $^{35}$S-methionine (700-1400 Ci/mmol); and then mixed with preincubated extract in a total volume of 0.1 ml at 37°C for 40 min. An equal volume of 2× SDS extraction buffer was added to stop the reaction, and the mixtures were placed in boiling water for 5 min. Three milliliters of cold acetone were added to each arrested assay mix, and the proteins were allowed to precipitate for 60 min at 4°C. The protein pellets were then collected by centrifugation at 5000 × g for 60 min, dried under vacuum and dissolved in SDS extraction buffer. Analysis of labeled proteins by SDS-gel electrophoresis was as described elsewhere (KARAM, MCCULLEY and LEACH 1977), except that fluorography (BONNER and LASKEY 1974), instead of standard autoradiography, was used to detect the in vitro synthesized $^{35}$S-labeled proteins.

Figure 2 shows the absorbance at 254 nm and translation profiles that were obtained in a typical fractionation and assay of RNA isolated from T4 regA$^-$-infected cells by using these methods. Most of the nucleic acid detected by absorbance measurements in such experiments is due to *E. coli* host transfer and ribosomal RNA species, which served as molecular size markers in our identification of phage-derived translational activities.

**RESULTS**

**Differential effects of T4 regA$^-$ mutations on stabilization of phage-induced transcripts:** Recently, YOUNG and MENARD (1981) demonstrated that T4-induced
mRNA populations can be fractionated according to size differences by preparative gel electrophoresis and identified by in vitro translation assays. Such fractionations exhibit a considerable degree of smearing in specific translational activity bands, probably due to a variety of factors including (1) overlapping modes of transcription for some phage cistrons, (2) metabolic instability of most T4-induced mRNAs, (3) possible mRNA processing events and (4) physical characteristics of the gel electrophoretic system used. The combination of these factors can generate mRNA elution profiles that are difficult to interpret especially when the transcripts of interest constitute minor components of the RNA pool. We used the T4 regA- mutation R9 to increase the relative abundance of gene 45 and gene 44 transcripts in phage-infected cells and to reduce the level of complexity of mRNA fractions in our analyses on these transcripts. T4 regA- mutations are known to specifically stabilize a subpopulation of T4-induced early mRNA species, including transcripts of the gene 45-44-62 cluster (Karam, McCulley and Leach 1977). Figure 3 shows some results of in vivo and in vitro translational activities derived from infections with T4 regA+ and regA- strains, respectively. In this experiment (Figure 3), infected cultures were treated with rifampicin at 8 min after infection and were then incubated in
the presence of the drug to allow degradation of unstable T4-induced RNA. RNA was subsequently purified from portions of the cultures and used for in vitro translations. The infected cultures were also analyzed for in vivo protein synthesis at the time of sampling for RNA extraction. As expected, RNA derived from T4 regA− infected cells exhibited much higher levels of in vitro protein synthesis than RNA from regA+ infections (Figure 3). In addition, several of the proteins that are known to be hyperproduced in regA− infections in vivo (WIBERG and KARAM 1983) were synthesized at high levels in vitro with regA− relative to regA+ derived mRNA. The results in Figure 3 also demonstrate that the translational activities for the T4 gene 44 and gene 45 proteins were particularly easy to assay under the conditions we used and, as will be shown, survived well the subsequent manipulations that were involved in fractionating T4-induced RNA by preparative gel electrophoresis.

Separation of the T4-induced transcripts for the gene 45 and 44 proteins by preparative gel electrophoresis: The results to be described in this section show that the T4 gene 45 protein and gene 44 protein are not encoded by one polycistronic mRNA. We utilized three classes of genotypes for the T4 gene 45-44 pair in our preparative gel electrophoretic analyses of T4-induced early mRNA populations: 45+44+ (wild-type alleles for the two genes), 45+44− (un-suppressed nonsense lesion in gene 44) and 45−44+ (un-suppressed nonsense lesion in gene 45). T4 32−rII B−42−regA− multiple mutants bearing the desired alleles for genes 45 and 44 were used to infect E. coli BÆ cultures, and at 8 min after infection, the cultures were treated with rifampicin to allow for in vivo degradation of those transcripts that are not subject to translational regulation by T4 regA protein. The regA−-stabilized phage-induced RNA species that remained after incubation with rifampicin (Figure 3) were fractionated on preparative gel electrophoretic columns, and the fractions were assayed for protein-synthesizing activity as described in MATERIALS AND METHODS. Representative results comparing the RNA translation profiles that we obtained with the three phage genotypes studied are shown in Figure 4. In these experiments (Figure 4), the T4-induced translational activity for gene 44 protein eluted in column fractions that overlapped with, but mostly preceded, the 23S ribosomal RNA marker. This activity was abolished in T4 44−(45+) infections and retained in 45− (44+) infections. By comparison, the translational activity for gene 45 protein, which was abolished in T4 45−(44+) and retained in T4 45+(44−) infections, eluted in fractions representing RNA species of much smaller size. From the results of several experiments similar to those shown in Figure 4, we estimated the gene 44 protein-encoding RNA species to be about 9.5 × 10^5 daltons (ca. 2.6 kb) and the gene 45 transcript to be about 3.3 × 10^5 daltons (ca. 0.9 kb). That is, whereas the gene 45 transcript seems to be of a size not much larger than is needed to encode the 227 amino acid gene 45 protein (SPICER and KONIGSBERG 1983), the gene 44 protein-synthesizing RNA appears to be abnormally large. Based on genetic data (KARAM, BOWLES and LEACH 1979; BOWLES and KARAM 1979), we suspect that T4 genes 44 and 62 are cotranscribed. The assays we used in the experiments for Figure 4 were too insensitive to detect in vitro synthesis of gene 62 protein. Neverthe-
FIGURE 4.—SDS-gel electrofluorograms showing the products of in vitro translation from electrophoretically fractionated RNA preparations of three E. coli Bφ cultures that were infected with T4 phage strains carrying the wild-type (44*45*), 44amE4408 (44*45*) and 45amE10 (44*45*) alleles for T4 genes 44 and 45, respectively. For each infection, the lane at the extreme left shows the 35S-labeled translation products from in vitro assays in which no exogenous RNA was added. The lanes marked C (extreme right) show translation products that were produced by the unfractionated RNAs. The positions of the gene 44 and 45 protein bands are indicated within the dashed zones of the figure. The vertical arrows indicate the positions of the peaks for 16S and 23S host ribosomal RNAs, which were determined spectrophotometrically. Experimental details are presented in MATERIALS AND METHODS.

less, genes 44 and 62 total about 1.5 kb in protein-encoding capacity as compared with the 2.6-kb size we estimate for the gene 44 protein-synthesizing RNA fractions (Figure 4). Conceivably, the gene 44/62 protein-encoding transcript also carries gene 45 nucleotide sequences that are either not translated or only poorly translated to gene 45 protein. We shall discuss this possibility further. We should also emphasize that our estimates of RNA sizes may be highly imprecise because we cannot yet predict the overall conformations of the RNA species that are detected by our column fractionations and in vitro translation assays.

DISCUSSION

A feature common to the chromosomes of many prokaryotes is the close linkage that exists between genes that specify related functions. Such genetic clustering is often related to coordinate control of transcription of DNA seg-
ments as, for example, in the regulation of bacterial operons and the temporal reading of the genomes of many bacteriophages in their infected bacterial hosts. Superimposed over the control of intracellular levels of gene products by transcriptional and translational mechanisms, there seems to be a relationship between genetic linkage and control of gene product interactions, i.e., control of the biological activities of proteins that function in multiprotein complexes. Stahl and Murray (1966) have suggested that clustering may provide a selective advantage by minimizing recombination between genes whose products must interact for biological function to ensue. Linkage arrangements may, with some functional clusters, also be related to intracellular compartmentalization of the assembly of multienzyme complexes. Two types of linkage-related effects on protein-protein assembly may be envisaged: (1) coupled translation of the cistrons in a polycistronic mRNA may position the newly synthesized polypeptides ideally for their interaction (Casjens and Hendrix 1974) and (2) chromosome folding at the site of coupled transcription and translation may bring polypeptides encoded by independently transcribed (and even unlinked) cistrons close together and minimize diffusion distances for proteins destined to interact. The interaction of T4 gene 45 protein with the gene 44/62 ATPase may represent an example of this latter type of compartmentalization. It has previously been shown that a nonsense lesion in T4 gene 45 (T4 45amElO) exerts a cis-dominant polar effect on the biological activity, but not on the synthesis, of a T4 gene 44 protein, a component of the gene 44/62 ATPase (Stahl et al. 1970; Karam, Bowles and Leach 1979). Based on our previous genetic and physiological studies (Bowles and Karam 1979; Karam, Bowles and Leach 1979) and on the studies reported here, it appears that the gene 45 and 44 proteins are not produced by the same polycistronic mRNA and that the polar effect of the T4 gene 45 lesion 45amElO on the gene 44 missense mutation, 44tsB110, is indeed an effect on the biological activity of a gene product (gene 44 protein) rather than its synthesis. Our results suggest that the nonsense fragment encoded by T4 45amElO in a non-suppressing (sup6) host poisons a specific ts peptide product (44tsB110) encoded by gene 44 but only when the two proteins are synthesized in close proximity to each other. Since the gene 45 and 44 transcripts are separable from each other, we propose that linkage at the DNA level can provide the compartmentalization needed for optimal interaction between proteins specified by two independently expressed T4 cistrons.

It should be emphasized that the sites for initiation of transcription of T4 genes 45, 44 and 62 have not yet been determined. In recent studies by Spicer and Konigsberg (1983) a large portion of the gene 45-44-62 cluster was sequenced at the DNA level. The sequencing data suggest that a transcriptional termination “loop” is located near the junction of genes 45 and 44 and that promoter-like sequences exist upstream of both genes 44 and 45 (see also Brody, Rabussay and Hall 1983). These observations are consistent with the separability of the gene 45 and gene 44 transcripts reported here. It should be noted, however, that the mRNA fractions that translate gene 44 protein in our experiments point to molecular species that are sufficiently large to include
more information than is needed to encode the gene 44 and 62 proteins. Our in vitro translation assays did not allow detection of gene 62 protein presumably because it is synthesized in a very small quantity (KARAM, BOWLES and LEACH 1979) and was masked by the high level of background synthesis of low-molecular weight peptides characteristic of such in vitro assays. Gene 45 protein synthesis, on the other hand, was detectable, but only in the small-molecular size RNA fractions (Figure 4). Small amounts of protein with molecular weight similar to that of gene 45 protein were detected in the translations of gene 44 mRNA fractions; however, the synthesis of these was not reduced in T4 45amE10 infections. Nevertheless, we cannot exclude the possibility that gene 45 is sometimes cotranscribed with the gene 44-62 pair and that the polycistronic species is inefficiently used for gene 45 protein synthesis. Alternatively, a polycistronic gene 45-44-62 transcript may be synthesized and rapidly cleaved (processed) to the separate species. Several reasons merit consideration of these possibilities: (1) the sensitivity of gene 44 function to UV-irradiation suggests that UV lesions in gene 45 are polar on gene 44 expression (HERCULES and SAUERBIER 1973), (2) two modes of gene 45 transcription have been suggested on the basis of UV-mapping data (HERCULES and SAUERBIER 1974) and (3) several T4 genes are known to be transcribed in two or more modes that yield different levels of translation for specific resident cistrons; examples include the T4 genes for lysozyme (BAUTZ et al. 1966; KASAI and BAUTZ 1969; JAYARAMA N and GOLDBERG 1970; CHRISTENSEN et al. 1984), the rIIb protein (SCHMIDT et al. 1970; SEDEROFF and EPSTEIN 1971; YOUNG and MENARD 1981) and the soc gene product (MacDONALD, KUTTER and MOSIG 1984). We are currently using cloned DNA fragments of the T4 gene 46-44-62-regA-43 cluster to probe and map the termini of its transcripts and to determine the genetic content of the large gene 44 transcript.

We are very grateful to Ted YOUNG and Rose MENARD for the extensive advice they provided in helping us learn to use the methods of RNA preparative gel electrophoresis and in vitro translation of phage-induced mRNA. We also thank MYRA DAWSON for help in the construction of the phage multiple mutants used in these studies. This work was supported by Public Health Service grant GM18842 from the National Institute of General Medical Sciences. Portions of this work were carried out by W. GERALD in partial fulfillment of the requirements for the M.S. degree at the College of Graduate Studies, Medical University of South Carolina, Charleston, 1980.

LITERATURE CITED


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